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ATTACHMENT 1

ORTH 345 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors : Patrick C. Kung and Gideon Goldstein

U.S. Patent No.: 4,361,549

Issued: : November 30, 1982

For : COMPLEMENT-FIXING MONOCLONAL ANTIBODY TO HUMAN T CELLS, AND METHODS OF PREPARING SAME

TRANSMITTAL LETTER

Hon. Commissioner of Patents and Trademarks
Box Patent EXT
Washington, D.C. 20231

Dear Sir:

Transmitted herewith is an Application for Extension of Patent Term under 35 U.S.C. 156 in the above-identified patent.

Please charge the $750.00 application fee to Deposit Account No. 10-750.

The Commissioner is hereby authorized to charge any additional fee which may be required or to credit any overpayments to Deposit Account No. 10-750.

A duplicate of this letter is enclosed.

C 10233 08/19/86 4361549 10-0750 010 111 750.00CH

RW10122 06/05/87 4361549 10-0750 010 111 200.00CR

Respectfully submitted,

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August 12 , 1986

Express Mail # B 11874864
Date of Deposit: August 12, 1986

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ORTH 345 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors : Patrick C. Kung and Gideon Goldstein

U.S. Patent No.: 4,361,549

Issued: : November 30, 1982

For : COMPLEMENT-FIXING MONOCLONAL ANTIBODY TO HUMAN T CELLS, AND METHODS OF PREPARING SAME

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. 156

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

Applicant, Ortho Pharmaceutical Corporation, a corporation of New Jersey, represents that it is the assignee of the entire interest in and to Letters Patent of the United States of America No. 4,361,549 granted to Patrick C. Kung and Gideon Goldstein on November 30, 1982, for COMPLEMENT-FIXING MONOCLONAL ANTIBODY TO HUMAN T CELLS, AND METHODS OF PREPARING SAME by virtue of an assignment to Ortho Pharmaceutical Corporation recorded in the United States Patent and Trademark Office on November 23, 1981, at reel 3928, frame 828.

Applicant hereby submits this Application for Extension of Patent Term under 35 U.S.C. 156 and provides the following information according to the guidelines published by the United States Patent and Trademark Office set forth in the Commissioner's Notice dated September 24, 1984, and appearing at 1047 OG 16-20 on October 9, 1984. The numbering of the following paragraphs correspond to the numbering of the requirements for an application set forth in Paragraph D(b) of the Commissioner's Notice.

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Date of Deposit: August 12, 1986

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(1)

The approved product is known by the generic name "MUROMONAB-CD3". The approved product is so described in the Product License ("PL") to which reference is made in paragraphs (9) and (10) below. The approved product is a murine monoclonal antibody to class CD 3 antigen.

(2)

The approved product was subject to regulatory review under the Public Health Service Act, Section 351 (42 U.S.C. 262).

(3)

The approved product received permission for commercial marketing or use under Section 351 of the Public Health Service Act (42 U.S.C. 262) on June 19, 1986.

(4)

This Application for Extension of Patent Term under 35 U.S.C. 156 is being submitted within the permitted 60 day period, which period will expire on August 18, 1986.

(5)

The complete identification of the patent for which extension of term is being sought is as follows:

Inventors: Patrick C. Kung and Gideon Goldstein

Patent No.: 4,361,549

Date of Issue: November 30, 1982

(6)

A complete copy of the patent identified in paragraph (5) and in the form specified is appended hereto as EXHIBIT A.

(7)

The records of the undersigned do not indicate any disclaimer, Certificate of Correction, receipt of maintenance fee payments, or reexamination certificates issued in the patent identified in paragraph (5). A complete copy of the Terminal Disclaimer dated July 1, 1981, and filed July 6, 1981, is appended hereto as EXHIBIT B. While this Terminal Disclaimer was filed, it never became effective since the earlier-filed application with respect to which it was filed (S.N. 22,132,filed March 20, 1979) issued as United States Patent No. 4,363,799 on December 14, 1982, which date is after November 30, 1982, the issue date of the patent identified in Paragraph (5).

(8)

United States Patent No. 4,361,549 claims the approved product. In particular, Claims 1, 2, 3, 4, 5, 7, 10 and 11 of U.S. Patent No. 4,361,549 claim and read on the approved product. With reference to Claims 1, 2, 3, 4, 5, 7, 10 and 11 of the EXHIBIT A copy of the patent, the claims read on the approved product as follows:

Claim 1: The approved product possesses the properties set out in claim 1 when measured as described in the specification.

Claim 2: The approved product is subclass Ig G_2.

Claim 3: The approved product is produced from a hybridoma formed by fusion of P3X63Ag-8U1 myeloma cells and spleen cells from a CAF_1 mouse previously immunized with E rosette purified human T cells.

Claim 4: The approved product is a complement-fixing monoclonal antibody of class Ig G produced by a hybridoma

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formed by fusion of cells from a mouse myeloma line and spleen cells from a mouse previously immunized with human T cells which reacts with essentially all normal human peripheral T cells but not with normal human peripheral B cells, null cells, or macrophages.

Claim 5: The approved product is a mouse complement-fixing monoclonal antibody which reacts with essentially all normal human peripheral T cells but not with normal human peripheral B cells, null cells, or macrophages.

Claim 7: The approved product is a complement-fixing monoclonal antibody which reacts with essentially all normal human peripheral T cells but not with normal human peripheral B cells, null cells, or macrophages prepared by the method which comprises the steps of:

(i) immunizing mice with E rosette positive purified human T cells;

(ii) removing the spleens from said mice and making a suspension of the spleen cells;

(iii) fusing said spleen cells with mouse myeloma cells in the presence of a fusion promoter;

(iv) diluting and culturing the fused cells in separate wells in a medium which will not support the unfused myeloma cells;

(v) evaluating the supernatant in each well containing a hybridoma for the presence of antibody to E rosette positive purified T cells;

(vi) selecting and cloning a hybridoma producing antibody which fixes complement and reacts with essentially all normal human peripheral T cells but not with normal human peripheral B cells, null cells, or macrophages;

(vii) transferring said clones intraperitoneally into mice; and

(viii) harvesting the malignant ascites or serum from said mice, which ascites or serum contains the desired antibody.

Claim 10: The approved product is produced by the method of claim 10.

Claim 11: The approved product is produced by the method of claim 10.

(9)

The relevant dates and information pursuant to 35 U.S.C. 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

(a) U.S. Patent No. 4,361,549 was issued on November 30, 1982.

(b) The Product License Application for the approved product was submitted on March 29, 1984, DHHS reference numbers 84-149 and 84-150.

(c) The Product License for the approved product was approved on June 19, 1986.

(10)

A brief description of the activities undertaken by Ortho Pharmaceutical Corporation during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities is appended hereto as EXHIBIT C.

Applicant is of the opinion that U.S. Patent 4,361,549 is eligible for extension under 35 U.S.C. 156 because it satisfies all of the requirements for such extension as follows:

i) 35 U.S.C. 156(a):

U.S. Patent 4,361,549 claims a product (claims 1, 2, 3, 4, 5, 7, and 11) and a method of making a product (Claim 10).

ii) 35 U.S.C. 156(a)(1):

The term of U.S. Patent 4,361,549 has not expired before submission of the present application.

iii) 35 U.S.C. 156(a)(2):

The term of U.S. Patent 4,361,549 has never been extended.

iv) 35 U.S.C. 156(a)(3):

The Application for Extension is submitted by the owner of record through its agent in accordance with the requirements of 35 U.S.C. 156(d).

v) 35 U.S.C. 156(a)(4):

The approved product has been subject to a regulatory review period before its commercial marketing or use.

vi) 35 U.S.C. 156(a)(5)(A):

The permission for the commercial marketing or use of the product after such regulatory review period is the first permitted commercial marketing or use of the product under the provision of the Public Health Service Act (42 U.S.C. 262) under which such regulatory review period occurred.

The length of extension of the patent term of U.S. Patent 4,361,549 requested by Applicant is two hundred one (201) days. The requested two hundred one day extension is the maximum permitted by the limitation of 35 U.S.C. 156(c)(3) since if granted it would extend the term of the patent to June 19, 2000, fourteen years from the date of approval of the approved product. The requested extension does not exceed the two-year maximum permitted by the limitation of 35 U.S.C. 156(g)(4)(C). This extension is supported by the regulatory review period for the approved product, which exceeds two (2) years. For the record, the regulatory review period as defined by 35 U.S.C. 156(g)(1)(B) is the total of: a) 1/2 of the applicable IND time under U.S.C. 156(g)(1)(B)(i), (not applicable in the present Application) and b) all of the PLA time, i.e., from March 29, 1984 until the date of approval for the approved product (June 19, 1986) or 812 days.

(12)

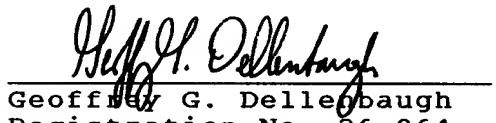
The Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to any determination to be made relative to the present Application for Extension.

(13)

ATTACHMENT 1 to this Application is a Transmittal Letter which requests that the required fee for the present Application for Extension be charged to Deposit Account No. 10-750. EXHIBIT E is a Declaration by the undersigned as agent for the Applicant as required by Paragraph (13) of the Guidelines.

Attached hereto as EXHIBIT D is a Power of Attorney and Appointment of Agents signed by an Assistant Secretary of the Applicant giving a Power of Attorney for the present Application for Extension to the undersigned, among others.

Respectfully submitted,



Geoffrey G. Dellenbaugh
Registration No. 26,864
Attorney for Applicants

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(201) 524-5545

CERTIFICATION

I hereby certify that this document as well as all attachments and exhibits thereto is being submitted in duplicate to the Commissioner of Patents and Trademarks, Box Patent EXT, Washington, D.C. 20231.

Date: August 12, 1986

Geoffrey G. Dellenbaugh

Johnson & Johnson
One Johnson & Johnson Plaza
New Brunswick, NJ 08933-7003
(201) 524-5545

STATE OF NEW JERSEY)
) ss.
COUNTY OF MIDDLESEX)

BE IT REMEMBERED, that on this 12th day of August, 1986, before me, a Notary Public, personally appeared Geoffrey G. Dellenbaugh, who I am satisfied is the person named in and who executed the foregoing instrument in my presence, and I having first made known to him the contents thereof, he did acknowledge that he signed, sealed, and delivered the same and his voluntary act and deed for the uses and purposed therein expressed.

Victoria L. Vodarsik
Notary Public

VICTORIA L. VODARSIK
NOTARY PUBLIC OF NEW JERSEY
My Commission Expires Nov. 28, 1989

9-5-86

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#23

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IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

Inventors : Patrick C. Kung and Gideon Goldstein

U.S. Patent No.: 4,361,549 SN 06-033669

Issued: : November 30, 1982

For : COMPLEMENT-FIXING MONOCLONAL ANTIBODY TO HUMAN T CELLS, AND METHODS OF PREPARING SAME

Express Mail Certificate

"Express Mail" mailing number B 11874864

Date of Deposit - August 12, 1986

I hereby certify that this complete Application for Extension of Patent Term including all Exhibits and Attachments named therein is being deposited in duplicate with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CRF 1.10 on the date indicated above and is addressed to the Commissioner of Patent and Trademarks, Box EXT, Washington, D.C. 20231.

Alwin M. Haywood
(Typed or printed name of person mailing paper or fee)

Alwin M. Haywood
(Signature of person mailing paper or fee)

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EXHIBIT A

United States Patent [19] [11] **4,361,549**
Kung et al. [45] **Nov. 30, 1982**

[54] **COMPLEMENT-FIXING MONOCLONAL ANTIBODY TO HUMAN T CELLS, AND METHODS OF PREPARING SAME**

[75] Inventors: **Patrick C. Kung**, Bridgewater; **Gideon Goldstein**, Short Hills, both of N.J.

[73] Assignee: **Ortho Pharmaceutical Corporation**, Raritan, N.J.

[21] Appl. No.: **33,669**

[22] Filed: **Apr. 26, 1979**

[51] **Int. Cl.**³ **C12Q 1/00**; G01N 33/48; G01N 33/68; G01N 33/96; A61K 39/395; C12N 5/00; C12N 5/02; C12N 15/00

[52] **U.S. Cl.** ..... **424/85**; 260/112 R; 424/8; 424/12; 424/101; 424/177; 435/7; 435/172; 435/240; 435/241

[58] **Field of Search** ..... 424/8, 112, 85, 89, 424/101, 177; 435/1,7, 172, 240, 241; 260/112 R, 112 B

[56] **References Cited**

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Primary Examiner—Anna P. Fagelson
Attorney, Agent, or Firm—Geoffrey G. Dellenbaugh

[57] **ABSTRACT**

Hybrid cell line for production of monoclonal antibody to an antigen found on all normal human T cells and cutaneous T lymphoma cells. The hybrid is formed by fusing splenocytes from immunized CAF_1 mice with P3X63Ag8Ul myeloma cells. Diagnostic and therapeutic uses of the monoclonal antibody are also disclosed.

11 Claims, 5 Drawing Figures

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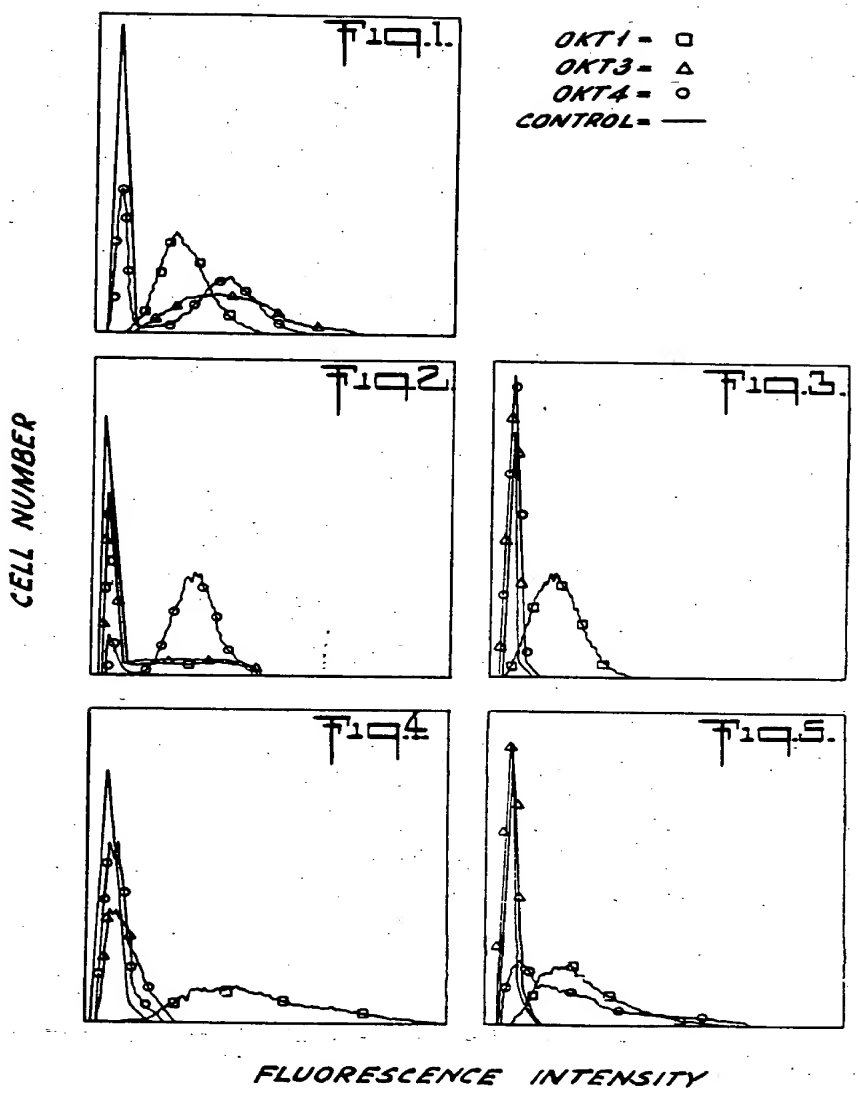
Fig.1.
OKT1 = □
OKT3 = △
OKT4 = ○
CONTROL = —
Fig.2.
Fig.3.
Fig.4.
Fig.5.
CELL NUMBER
FLUORESCENCE INTENSITY

COMPLEMENT-FIXING MONOCLONAL ANTIBODY TO HUMAN T CELLS, AND METHODS OF PREPARING SAME

FIELD OF THE INVENTION

This invention relates generally to new hybrid cell lines and more specifically to hybrid cell lines for production of complement-fixing monoclonal antibody to an antigen found on all normal human T cells and cutaneous T lymphoma cells, to the antibody so produced, and to therapeutic and diagnostic methods and compositions employing this antibody.

DESCRIPTION OF THE PRIOR ART

The fusion of mouse myeloma cells to spleen cells from immunized mice by Kohler and Milstein in 1975 [Nature 256, 495–497 (1975)] demonstrated for the first time that it was possible to obtain a continuous cell line making homogeneous (so-called "monoclonal") antibody. Since this seminal work, much effort has been directed to the production of various hybrid cells (called "hybridomas") and to the use of the antibody made by these hybridomas for various scientific investigations. See, for example, *Current Topics in Microbiology and Immunology*, Volume 81-"Lymphocyte Hybridomas", F. Melchers, M. Potter, and N. Warner, Editors, Springer-Verlag, 1978, and references contained therein; C. J. Barnstable, et al., Cell, 14, 9–20 (May, 1978); P. Parham and W. F. Bodmer, *Nature* 276, 397–399 (November, 1978); *Handbook of Experimental Immunology*, Third Edition, Volume 2; D. M. Wier, Editor, Blackwell, 1978, Chapter 25; and *Chemical and Engineering News*, Jan. 1, 1979, 15–17. These references simultaneously indicate the rewards and complications of attempting to produce monoclonal antibody from hybridomas. While the general technique is well understood conceptually, there are many difficulties met and variations required for each specific case. In fact, there is no assurance, prior to attempting to prepare a given hybridoma, that the desired hybridoma will be obtained, that it will produce antibody if obtained, or that the antibody so produced will have the desired specificity. The degree of success is influenced principally by the type of antigen employed and the selection technique used for isolating the desired hybridoma.

The attempted production of monoclonal antibody to human lymphocyte cell-surface antigens has been reported only in a few instances. See, for example, *Current Topics in Microbiology and Immunology*, ibid, 66–69 and 164–169. The antigens used in these reported experiments were cultured human lymphoblastoid leukemia and human chronic lymphocytic leukemia cell lines. Many hybridomas obtained appeared to produce antibody to various antigens on all human cells. None of the hybridomas produced antibody against a predefined class of human lymphocytes.

It should be understood that there are two principal classes of lymphocytes involved in the immune system of humans and animals. The first of these (the thymus-derived cell or T cell) is differentiated in the thymus from haemopoietic stem cells. While within the thymus, the differentiating cells are termed "thymocytes." The mature T cells emerge from the thymus and circulate between the tissues, lymphatics, and the bloodstream. These T cells form a large proportion of the pool of recirculating small lymphocytes. They have immunological specificity and are directly involved in cell-me-

diated immune responses (such as graft rejection) as effector cells. Although T cells do not secrete humoral antibodies, they are sometimes required for the secretion of these antibodies by the second class of lymphocytes discussed below. Some types of T cells play a regulating function in other aspects of the immune system. The mechanism of this process of cell cooperation is not yet completely understood.

The second class of lymphocytes (the bone marrow-derived cells or B cells) are those which secrete antibody. They also develop from haemopoietic stem cells, but their differentiation is not determined by the thymus. In birds, they are differentiated in an organ analogous to the thymus, called the Bursa of Fabricius. In mammals, however, no equivalent organ has been discovered, and it is thought that these B cells differentiate within the bone marrow.

It is now recognized that T cells are divided into at least several subtypes, termed "helper", "suppressor", and "killer" T cells, which have the function of (respectively) promoting a reaction, suppressing a reaction, or killing (lysing) foreign cells. These subclasses are well understood for murine systems, but they have only recently been described for human systems. See, for example, R. L. Evans, et al., *Journal of Experimental Medicine,* Volume 145, 221–232, 1977; and L. Chess and S. F. Schlossman—"Functional Analysis of Distinct Human T-Cell Subsets Bearing Unique Differentiation Antigens", in "*Contemporary Topics in Immunobiology*", O. Stutman, Editor, Plenum Press, 1977, Volume 7, 363–379.

The ability to identify or suppress classes or subclasses of T cells is important for diagnosis or treatment of various immunoregulatory disorders or conditions.

For example, certain leukemias and lymphomas have differing prognosis depending on whether they are of B cell or T cell orgin. Thus, evaluation of the disease prognosis depends upon distinguishing between these two classes of lymphocytes. See, for example, A. C. Aisenberg and J. C. Long, *The American Journal of Medicine,* 58:300 (March, 1975); D. Belpomme, et al., in "*Immunological Diagnosis of Leukemias and Lymphomas*", S. Thierfelder, et al., eds, Springer, Heidelberg, 1977, 33–45; and D. Belpomme, et al., *British Journal of Haematology,* 1978, 38, 85. Certain disease states (e.g., juvenile rheumatoid arthritis and certain leukemias) are associated with an imbalance of T cell subclasses. It has been suggested that autoimmune diseases generally are associated with an excess of "helper" T cells or a deficiency of certain "suppressor" T cells, while malignancies generally are associated with an excess of "suppressor" T cells. In certain leukemias, excess T cells are produced in an arrested stage of development. Diagnosis may thus depend on the ability to detect this imbalance or excess. See, for example, J. Kersey, et al., "Surface Markers Define Human Lymphoid Malignancies with Differing Prognoses" in *Haematology and Blood Transfusion,* Volume 20, Springer-Verlag, 1977, 17–24, and references contained therein.

On the therapeutic side, there is some suggestion, as yet not definitely proven, that administration of antibodies against the subtype of T cell in excess may have therapeutic benefit in autoimmune disease or malignancies. Antisera against the entire class of human T cells (so-called antihuman thymocyte globulin or ATG) has been reported useful therapeutically in patients receiving organ transplants. Since the cell-mediated immune

response (the mechanism whereby transplants are rejected) depends upon T cells, administration of antibody to T cells prevents or retards this rejection process. See, for example, Cosimi, et al., "Randomized Clinical Trial of ATG in Cadaver Renal Allgraft Recipients: Importance of T Cell Monitoring", *Surgery* 40:155–163 (1976) and references contained therein.

The identification and suppression of human T cell classes and subclasses has previously been accomplished by the use of spontaneous autoantibodies or selective antisera for human T cells obtained by immunizing animals with human T cells, bleeding the animals to obtain serum, and adsorbing the antiserum with (for example) autologous but not allogeneic B cells to remove antibodies with unwanted reactivities. The preparation of these antisera is extremely difficult, particularly in the adsorption and purification steps. Even the adsorbed and purified antisera contain many impurities in addition to the desired antibody, for several reasons. First, the serum contains millions of antibody molecules even before the T cell immunization. Second, the immunization causes production of antibodies against a variety of antigens found on all human T cells injected. There is no selective production of antibody against a single antigen. Third, the titer of specific antibody obtained by such methods is usually quite low, (e.g., inactive at dilutions greater than 1:100) and the ratio of specific to non-specific antibody is less than $1/10^6$.

See, for example, the Chess and Schlossman article referred to above (at pages 365 and following) and the Chemical and Engineering News article referred to above, where the deficiencies of prior art antisera and the advantages of monoclonal antibody are described.

SUMMARY OF THE INVENTION

There has now been discovered a noval hybridoma (designated OKT3) which is capable of producing novel complement-fixing monoclonal antibody against an antigen found on essentially all normal human peripheral T cells and cutaneous T lymphoma cells. The antibody so produced is mono-specific for a single determinant on normal human T cells and cutaneous T lymphoma cells and contains essentially no other anti-human immuneglobulin, in contrast to prior art antisera (which are inherently contaminated with antibody reactive to numerous human antigens) and to prior art monoclonal antibodies (which are not monospecific for a human T cell antigen). Moreoever, this hybridoma can be cultured to produce antibody without the necessity of immunizing and killing animals, followed by the tedious adsorption and purification steps necessary to obtain even the impure antisera of the prior art.

It is accordingly one object of this invention to provide hybridomas which produce antibodies against an antigen found on essentially all normal human T cells and cutaneous T lymphoma cells.

It is a further aspect of the present invention to provide methods for preparing these hybridomas.

A further object of the invention is to provide essentially homogeneous antibody against an antigen found on essentially all normal human T cells and cutaneous T lymphoma cells.

A still further object is to provide methods for treatment or diagnosis of disease employing these antibodies.

Other objects and advantages of the invention will become apparent from the examination of the present disclosure.

In satisfaction of the foregoing objects and advantages, there is provided by this invention a novel hybridoma producing novel antibody to an antigen found on essentially all normal human T cells and cutaneous T lymphoma cells, the antibody itself, and diagnostic and therapeutic methods employing the antibody. The hydbridoma was prepared generally following the method of Milstein and Kohler. Following immunization of mice with normal E rosette positive human T cells, the spleen cells of the immunized mice were fused with cells from a mouse myeloma line and the resultant hybridomas screened for those with supernatants containing antibody which gave selective binding to normal E rosette positive human T cells. The desired hybridomas were subsequently cloned and characterized. As a result, a hybridoma was obtained which produces antibody (designated OKT3) against an antigen on essentially all normal human T cells. Not only does this antibody react with essentially all normal human peripheral T cells, but it also does not react with other normal peripheral blood lymphoid cells. In addition, the cell surface antigen recognized by this antibody is detected on only mature thymocytes and is completely lacking on greater than 90% of normal human thymocytes.

In view of the difficulties indicated in the prior art and the lack of success reported using malignant cell lines as the antigen, it was surprising that the present method provided the desired hybridoma. It should be emphasized that the unpredictable nature of hybrid cell preparation does not allow one to extrapolate from one antigen or cell system or another. In fact, the present applicants have discovered that using a T cell malignant cell line as the antigen caused formation of hybridomas which did not produce the desired antibody. Attempts to use purified antigens separated from the cell surfaces were also unsuccessful.

Both the subject hybridoma and the antibody produced thereby are identified herein by the designation "OKT3", the particular material referred to being apparent from the context. The subject hybridoma was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852 on Apr. 26, 1979, and assigned the ATCC accession number CRL 8001.

The preparation and characterization of the hybridoma and the resultant antibody will be better understood by reference to the following description and Examples.

DETAILED DESCRIPTION OF THE INVENTION

The method of preparing the hybridoma generally comprises the following steps:

A. Immunizing mice with E rosette positive purified normal human peripheral T cells. While it has been found that female CAF_1 mice (a first generation hybrid between Balb/cJ and A/J mice) are preferred, it is contemplated that other mouse strains could be used. The immunization schedule and T cell concentration should be such as to produce useful quantities of suitably primed splenocytes. Three immunizations at fourteen day intervals with 2×10^7 cells/mouse/injection in 0.2 ml phosphate buffered saline has been found to be effective.

B. Removing the spleens from the immunized mice and making a spleen suspension in an appropriate medium. About one ml of medium per spleen is sufficient. These experimental techniques are well-known.

C. Fusing the suspended spleen cells with mouse myeloma cells from a suitable cell line by the use of a suitable fusion promoter. The preferred ratio is about 5 spleen cells per myeloma cell. A total volume of about 0.5–1.0 ml of fusion medium is appropriate for about 10^8 splenocytes. Many mouse myeloma cell lines are known and available, generally from members of the academic community or various deposit banks, such as the Salk Institute Cell Distribution Center, La Jolla, CA. The cell line used should preferably be of the so-called "drug resistant" type, so that unfused myeloma cells will not survive in a selective medium, while hybrids will survive. The most common class is 8-azaguanine resistant cell lines, which lack the enzyme hypoxanthine gaunine phophoribosyl transferase and hence will not be supported by HAT (hypoxanthine, aminopterin, and thymidine) medium. It is also generally preferred that the myeloma cell line used be of the so-called "non-secreting" type, in that it does not itself produce any antibody, although secreting types may be used. In certain cases, however, secreting myeloma lines may be preferred. While the preferred fusion promoter is polyethylene glycol having an average molecular weight from about 1000 to about 4000 (commercially available as PEG 1000, etc.), other fusion promoters known in the art may be employed.

D. Diluting and culturing in separate containers, the mixture of unfused spleen cells, unfused myeloma cells, and fused cells in a selective medium which will not support the unfused myeloma cells for a time sufficient to allow death of the unfused cells (about one week). The dilution may be a type of limiting one, in which the volume of diluent is statistically calculated to isolate a certain number of cells (e.g., 1–4) in each separate container (e.g., each well of a microtiter plate). The medium is one (e.g., HAT medium) which will not support the drug-resistant (e.g., 8-azaguanine resistant) unfused myeloma cell line. Hence, these myeloma cells perish. Since the unfused spleen cells are non-malignant, they have only a finite number of generations. Thus, after a certain period of time (about one week) these unfused spleen cells fail to reproduce. The fused cells, on the other hand, continue to reproduce because they possess the malignant quality of the myeloma parent and the ability to survive in the selective medium of the spleen cell parent.

E. Evaluating the supernatant in each container (well) containing a hybridoma for the presence of antibody to E rosette positive purified human T cells.

F. Selecting (e.g., by limiting dilution) and cloning hybridomas producing the desired antibody.

Once the desired hybridoma has been selected and cloned, the resultant antibody may be produced in one of two ways. The purest monoclonal antibody is produced by in vitro culturing of the desired hybridoma in a suitable medium for a suitable length of time, followed by recovery of the desired antibody from the supernatant. The suitable medium and suitable length of culturing time are known or are readily determined. This in vitro technique produces essentially monospecific monoclonal antibody, essentially free from other specific antihuman immune globulin. There is a small amount of other immune globulin present since the medium contains xenogeneic serum (e.g., fetal calf serum). However, this in vitro method may not produce a sufficient quantity or concentration of antibody for some purposes, since the concentration of monoclonal antibody is only about 50 μg/ml.

To produce a much greater concentration of slightly less pure monoclonal antibody, the desired hybridoma may be injected into mice, preferably syngenic or semi-syngenic mice. The hybridoma will cause formation of antibody-producing tumors after a suitable incubation time, which will result in a high concentration of the desired antibody (about 5–20 mg/ml) in the blood-stream and peritoneal exudate (ascites) of the host mouse. Although these host mice also have normal antibodies in their blood and ascites, the concentration of these normal antibodies is only about 5% of the monoclonal antibody concentration. Moreover, since these normal antibodies are not antihuman in their specificity, the monoclonal antibody obtained from the harvested ascites or from the serum is essentially free of any contaminating antihuman immune globulin. This monoclonal antibody is high titer (active at dilutions of 1:100,000 or higher) and high ratio of specific to non-specific immune globulin (about 1/20). Immune globulin produced incorporating the κ light myeloma chains are non-specific, "nonsense" peptides which merely dilute the monoclonal antibody without detracting from its specificity.

EXAMPLE I

Production of Monoclonal Antibodies

A. Immunization and Somatic Cell Hybridization

Female CAF_1 mice (Jackson Laboratories; 6–8 weeks old) were immunized intraperitoneally with 2×10^7 E rosette purified T cells in 0.2 ml of phosphate buffered saline at 14-day intervals. Four days after the third immunization, spleens were removed from the mice, and a single cell suspension was made by pressing the tissue through a stainless steel mesh.

Cell fusion was carried out according to the procedure developed by Kohler and Milstein. 1×10^8 splenocytes were fused in 0.5 ml of a fusion medium comprising 35% polyethylene glycol (PEG 1000) and 5% dimethylsulfoxide in RPMI 1640 medium (Gibco, Grand Island, NY) with 2×10^7 P3X63Ag8U1 myeloma cells supplied by Dr. M. Scharff, Albert Einstein College of Medicine, Bronx, NY. These myeloma cells secrete IgG_1 κ light chains.

B. Selection and Growth of Hybridoma

After cell fusion, cells were cultured in HAT medium (hypoxanthine, aminopterin, and thymidine) at 37° C. with 5% CO_2 in a humid atmosphere. Several weeks later, 40 to 100 μl of supernatant from cultures containing hybridomas were added to a pellet of 10^6 peripheral lymphocytes separated into E rosette positive (E^+) and E rosette negative (E^-) populations, which were prepared from blood of healthy human donors as described by Mendes (*J. Immunol.* 111:860, 1973). Detection of mouse hybridoma antibodies binding to these cells was determined by radioimmunoassay and/or indirect immunofluorescence. In the first method, the cells were initially reacted with 100 μl of affinity-purified ^{125}I goat-anti-mouse IgG (10^6 cpm/μg; 500 μg/μl). (Details of iodination of goat-anti-mouse IgG were described by Kung, et al., *J. Biol. Chem.* 251(8):2399, 1976). Alternatively, cells incubated with culture supernatants were stained with a fluorescinated goat-anti-mouse IgG (G/M FITC) (Meloy Laboratories, Springfield, VA; F/p=2.5) and the fluorescent antibody-coated cells were subsequently analyzed on the Cytofluorograf FC200/4800A (Ortho Instruments, Westwood, MA) as described in Example III. Hybridoma cultures contain-

ing antibodies reacting specifically with E+ lymphocytes (T cells) were selected and cloned. Subsequently, the clones were transferred intraperitoneally by injecting 1×10^7 cells of a given clone (0.2 ml volume) into CAF_1 mice primed with 2,6,10,14-tetramethylpentadecane, sold by Aldrich Chemical Company under the name Pristine. The malignant ascites from these mice were then used to characterize lymphocytes as described below in Example II. The subject hybrid antibody OKT3 was demonstrated by standard techniques to be of IgG_2 subclass and to fix complement.

EXAMPLE II

Characterization of OKT3 Reactivity

A. Isolation of Lymphocyte Populations

Human peripheral blood mononuclear cells were isolated from healthy volunteer donors (ages 15–40) by Ficoll-Hypaque density gradient centrifugation (Pharmacia Fine Chemicals, Piscataway, NJ) following the technique of Boyum, *Scand. J. Clin. Lab. Invest.* 21 (Suppl. 97): 77, 1968. Unfractionated mononuclear cells were separated into surface Ig+ (B) and Ig− (T plus Null) populations by Sephadex G-200 anti-$F(ab')_2$ column chromatography as previously described by Chess, et al., *J. Immunol.* 113:1113 (1974). T cells were recovered by E rosetting the Ig− population with 5% sheep erythrocytes (Microbiological Associates, Bethesda, MD). The rosetted mixture was layered over Ficoll-Hypaque and the recovered E+ pellet treated with 0.155 M NH_4Cl (10 ml per 10^8 cells). The T cell population so obtained was <2% EAC rosette positive and >95% E rosette positive as determined by standard methods. In addition, the non-rosetting Ig− (Null cell) population was harvested from the Ficoll interface. This latter population was <5% E+ and ≦2% sIg+. The surface Ig+ (B) population was obtained from the Sephadex G-200 column following elution with normal human gamma globulin as previously described. This population was >95% surface Ig+ and <5% E+.

Normal human macrophages were obtained from the mononuclear population by adherence to polystyrene. Thus, mononuclear cells were resuspended in final culture media (RPMI 1640, 2.5 mM HEPES [4-(2-hydroxyethyl)-1-piperazinepropane sulfonic acid] buffer, 0.5% sodium bicarbonate, 200 mM L-glutamine, and 1% penicillin-streptomycin, supplemented with 20% heat-inactivated human AB serum) at a concentration of 2×10^6 cells and incubated in plastic petri dishes (100×20 mm) (Falcon Tissue Culture Dish; Falcon, Oxnard, CA) at 37° C. overnight. After extensive washing to remove non-adherent cells, the adherent population was detached by brisk washing with cold serum-free medium containing 2.5 mM EDTA and occasional scraping with the rubber tip of a disposable syringe plunger. Greater than 85% of the cell population was capable of ingesting latex particles and had morphologic characteristics of monocytes by Wright-Giemsa staining.

B. Normal Thymus

Normal human thymus gland was obtained from patients aged two months to 14 years undergoing corrective cardiac surgery. Freshly obtained portions of the thymus gland were immediately placed in 5% fetal calf serum in medium 199 (Gibco), finely minced with forceps and scissors, and subsequently made into single cell suspensions by being pressed through wire mesh. The cells were next layered over Ficoll-Hypaque and spun and washed as previously described in section A

above. The thymocytes so obtained were >95% viable and ≧90% E rosette positive.

C. Cell Lines

Epstein-Barr Virus (EBV) transformed B cell lines from four normal individuals (Laz 007, Laz 156, Laz 256, and SB) and described. T cell lines CEM, HJD-1, Laz 191, and HM1 established from leukemic patients were provided by Dr. H. Lazarus, Sidney Farber Cancer Institute, Boston, MA.

D. T Acute Lymphoblastic Leukemia (T-ALL) Cells and T Chronic Lymphatic Leukemia (T-CLL) Cells

Leukemia cells were obtained from 12 patients with T-ALL. These individuals' cells had previously been determined to be of T cell lineage by their spontaneous rosette formation with sheep erythrocytes (>20% E+) and reactivity with T cell specific hetero-antisera, anti-HTL (anti-B.K.) and A99, as previously described by Schlossman, et al., *Proc. Nat. Acad. Sci.* 73:1288 (1976). Tumor cells from three individuals were reactive (TH_2+) with rabbit and/or equine anti-TH_2 while cells from the remaining nine were non-reactive (TH_2−). Leukemic cells from two patients with TH_2− T-CLL were also utilized. Both acute and chronic T cell leukemia cells were cryopreserved in −196° C. vapor phase liquid nitrogen in 10% dimethylsulfoxide and 20% AB human serum until the time of surface characterization. The tumor populations analyzed were >90% blasts by Wright-Giemsa morphology in all instances.

EXAMPLE III

Cytofluorographic Analysis

Cytofluorographic analysis of all cell populations was performed by indirect immunofluorescence with fluorescein-conjugated goat-anti-mouse IgG (G/M FITC) (Meloy Laboratories) on a Cytofluorograf FC200/4800A (Ortho Instruments). In brief, $1-2\times10^6$ cells were treated with 0.15 ml OKT3 at a 1:1000 dilution, incubated at 4° C. for 30 minutes, and washed twice. The cells were then reacted with 0.15 ml of a 1:40 dilution G/M FITC at 4° C. for 30 minutes, centrifuged, and washed three times. These cells were then analyzed on the Cytofluorograf and the intensity of fluorescence per cell recorded on a pulse height analyzer. A similar pattern of reactivity was observed at a dilution of 1:100,000, but further dilution caused loss of reactivity. Background staining was obtained by substituting a 0.15 ml aliquot of 1:1000 ascites from a Balb/cJ mouse intraperitoneally immunized with a non-producing hybrid clone.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the fluorescence pattern obtained on the Cytofluorograf after reacting normal human peripheral T cells with OKT3 at a 1:1000 dilution and G/M FITC. For comparison, results with monoclonal antibodies OKT1 and OKT4 are shown under equivalent conditions in FIGS. 1-5.

FIG. 2 shows the fluorescence pattern obtained on the Cytofluorograf after reacting human thymocytes with OKT3 and G/M FITC.

FIG. 3 shows the fluorescence pattern obtained on the Cytofluorograf after reacting leukemic cells from B cell chronic lymphoblastic leukemia patients with OKT3 and G/M FITC.

FIG. 4 shows the fluorescence pattern obtained on the Cytofluorograf after reacting the human T cell line HJD-1 with OKT3 and G/M FITC.

FIG. 5 shows the fluorescence pattern obtained on the Cytofluorograf after reacting the human T cell line CEM with OKT3 and G/M FITC.

The data in FIGS. 1–5 plus additional data for OKT3 (as well as OKT1 and OKT4) are summarized in Table I.

The production of the hybridoma and the production and characterization of the resulting monoclonal antibody were conducted as described in the above Examples. Although large quantities of the subject antibody were prepared by injecting the subject hybridoma intraperitoneally into mice and harvesting the malignant ascites, it is clearly contemplated that the hybridoma could be cultured in vitro by techniques well-known in the art and the antibody removed from the supernatant.

As shown in FIG. 1, the entire human peripheral blood T cell population of a given normal individual is reactive with OKT3, whereas the entire B cell, null cell, and macrophage populations isolated from the same individual are unreactive with OKT3. Similar results were obtained on populations of lymphocytes from fifteen other normal individuals. The monoclonal antibody is thus characterized in that it is reactive with an antigen contained on the surface of essentially all normal human peripheral T cells, while being unreactive with any antigens on the surface of the other three cell types discussed above. This differential reactivity is one test by which the subject antibody OKT3 may be detected and distinguished from other antibodies.

As shown in FIG. 2, the vast majority of normal human thymocytes from a six-month old infant are completely unreactive with OKT3, while about 5 to 10 percent of the thymocytes are reactive. The implication of this finding is that, during the differentiation process by which stem cells are converted into mature T cells, the thymocytes acquire at some stage the same surface antigen found on T cells, which is reactive with OKT3. It is believed that these thymocytes are in the later stages of differentiation just prior to emergence from the thymus into the bloodstream. Similar results (5–10% reactivity) were obtained using six additional thymus specimens from normal individuals two months to 19 years of age. The pattern of reactivity in FIG. 2 provides a second method of detecting the subject antibody OKT3 and distinguishing it from other antibodies.

The subject antibody is also useful for determining the proportion of circulating lymphocytes that are T cells. As shown in Table I, ≧95% of all T cells react with OKT3 antibody. The present invention thus includes a method for determining in an individual the proportion of circulating lymphocytes that are T cells which comprises mixing OKT3 antibody with a lymphocyte composition from the individual and determining the preparation of the lymphocytes which are OKT3+, and thus T cells.

A further characterization of the subject antibody OKT3 is shown by the reactivity to various human T cell lines illustrated in FIGS. 4 and 5. As can be seen, the reactivity of the subject antigen to human T cell lines was heterogeneous, being weak for the line HJD-1, and nonexistent for the lines CEM, Laz 191, and HM1. This differential reactivity of OKT3 to various readily-available human T cell lines provides yet another method of characterizing and describing the subject antibody.

The lack of reaction of OKT3 with the human B cell lines Laz 007, Laz 156, Laz 256, and SB is shown in Table I. This further supports the lack of reactivity of

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OKT3 with B cells obtained from the peripheral blood of a normal human population and provides yet another method for characterizing and distinguishing the subject antibody OKT3.

The specific reaction of OKT3 antibody with an antigen on cutaneous T cell lymphomas is illustrated by Table II, where the distinction from OKT1 and OKT4 is shown. The present antibody thus provides a reagent for confirming a diagnosis of cutaneous T cell lymphoma in a patient suspected of having said disease. Treatment of cutaneous T cell lymphoma by administration of a therapeutically effective amount of OKT3 antibody is also contemplated as part of the present invention.

According to the present invention there are provided a hybridoma capable of producing antibody against an antigen found on essentially all normal human T cells and cutaneous T lymphoma cells, a method for producing this hybridoma, monoclonal antibody against an antigen found on essentially all human T cells, methods for producing the antibody, and methods for treatment or diagnosis of disease employing this antibody.

Although only a single hybridoma producing a single monoclonal antibody against human T cell antigen is described, it is contemplated that the present invention encompasses all monoclonal antibodies exhibiting the characteristics described herein. It was determined that the subject antibody OKT3 belongs to the subclass IgG_2, which is one of four subclasses of murine IgG. These subclasses of immune globulin G differ from one another in the so-called "fixed" regions, although an antibody to a specific antigen will have a so-called "variable" region which is functionally identical regardless of which subclass of immune globulin G it belongs to. That is, a monclonal antibody exhibiting the characteristic described herein may be of subclass IgG_1, IgG_2a, IgG_2b, or IgG_3, or of classes IgM, IgA, or other known Ig classes. The differences among these classes or subclasses will not affect the selectivity of the reaction pattern of the antibody, but may affect the further reaction of the antibody with other materials, such as (for example) complement or anti-mouse antibodies. Although the subject antibody is specifically IgG_2, it is contemplated that antibodies having the patterns of reactivity illustrated herein are included within the subject invention regardless of the immune globulin class or subclass to which they belong.

Further included within the subject invention are methods for preparing the monoclonal antibodies described above employing the hybridoma technique illustrated herein. Although only one example of a hybridoma is given herein, it is contemplated that one skilled in the art could follow the immunization, fusion, and selection methods provided herein and obtain other hybridomas capable of producing antibodies having the reactivity characteristics described herein. Since the individual hybridoma produced from a known mouse myeloma cell line and spleen cells from a known species of mouse cannot be further identified except by reference to the antibody produced by the hybridoma, it is contemplated that all hybridomas producing antibody having the reactivity characteristics described above are included within the subject invention, as are methods for making this antibody employing the hybridoma.

Further aspects of the invention are methods of treatment or diagnosis of disease employing the monoclonal antibody OKT3 or any other monoclonal antibody

exhibiting the pattern of reactivity provided herein. As discussed above, the subject antibody allows treatment of patients having certain T cell chronic lymphoblastic leukemias by administration of a therapeutically-effective amount thereof. Administration of a therapeutically-effective amount of OKT3 antibody to an individual subject undergoing organ transplant will reduce or eliminate the rejection of this transplant. The subject antibody also allows detection of cutaneous T cell lymphoma in an individual by mixing a lymphoma T cell composition from said individual with a diagnostically-effective amount of OKT3 antibody. The presence of a reaction confirms the identity of the disease. The cutaneous T cell lymphoma may be treated by administering to an individual in need of such treatment a therapeutically-effective amount of OKT3 antibody. This antibody will react with and reduce the amount of T lymphoma cells, thus ameliorating the disease. In view of these diagnostic and therapeutic methods, the present invention additionally includes diagnostic and therapeutic compositions comprising (respectively) a diagnostically-effective or therapeutically-effective amount of OKT3 antibody in a diagnostically or pharmaceutically acceptable carrier.

TABLE I

MONOCLONAL ANTIBODY REACTIVITY AND PROPERTIES

	Monoclonal Antibodies		
	OKT1	OKT3	OKT4
% Reactivity With:			
Peripheral T-cells (10 samples)	>95%	>95%	55%
Peripheral B-cells (10 samples)	<2%	<2%	<2%
Peripheral Null cells (10 samples)	<2%	<2%	<2%
Thymocytes* (8 samples)	5–10%	5–10%	80%
Reactivity With:			
T-chronic lymphatic Leukemia (3 cases)	+	+(1);−(2)	−
T-acute lymphatic Leukemia (8 cases)	−	−	−
Null acute lymphatic Leukemia (15 cases)	−	−	−
B-chronic lymphatic Leukemia (6 cases)	+(4);−(2)	−	−
B-cell lines+ (4)	−	−	−
T-cell lines+ HJD-1	+	(±)	−
CEM	+	−	+
Laz 191	+	−	−
HMI	+	−	−
IgG Subclass	IgG_1	IgG_2	IgG_2
Complement fixation	−	+	+

*From patients aged 2 months to 18 years
+Obtained from Dr. H. Lazarus, Sidney Farber Cancer Center. B cell lines Laz 256, 156, 007 and SB obtained by Epstein-Barr virus transformation of human peripheral B cells and HJD-1, CEM, Laz 191, and HMI established from leukemia patients.

TABLE II

Patient's Name	Cutaneous T-Cell Lymphoma DIAGNOSIS	MONOCLONAL ANTIBODY ASSAYS OKT1	OKT3	OKT4
E. McBride	Sezary Blast Crisis; PBL	+	+	−
C. O. Okley	Mycosis Fungoides; Node	−	+	+
Odom	Mycosis Fungoides; Node	+	+	−
Montalbono	? Node	+	+	+

Source of cells:
PBL = peripheral blood lymphocytes
Node = lymph node

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What is claimed is:

1. A monoclonal antibody of class IgG produced by a hybridoma formed by fusion of cells from a mouse myeloma line and spleen cells from a mouse previously immunized with human T cells, which antibody:

(a) reacts with essentially all normal human peripheral T cells and cutaneous T lymphoma cells, but not with normal human peripheral B cells, null cells or macrophages;

(b) reacts with from about 5% to about 10% of normal human thymocytes;

(c) reacts with leukemic cells from humans with T cell chronic lymphoblastic leukemia but does not react with leukemic cells from humans with T cell acute lymphoblastic leukemia, null cell acute lymphoblastic leukemia, or B cell chronic lymphatic leukemia;

(d) reacts weakly with the human T cell line HJD-1 but does not react with CEM, Laz 191, or HMl;

(e) does not react with the Epstein-Barr virus-transformed human B cell lines Laz 007, Laz 156, Laz 256, or SB; and

(f) fixes complement.

2. The monoclonal antibody of claim 1 which is of subclass IgG_2.

3. The monoclonal antibody of claim 1 which is produced from a hybridoma formed by fusion of P3X63Ag-8U1 myeloma cells and spleen cells from a CAF_1 mouse previously immunized with E rosette purified human T cells.

4. A complement-fixing monoclonal antibody of class IgG produced by a hybridoma formed by fusion of cells from a mouse myeloma line and spleen cells from a mouse previously immunized with human T cells which reacts with essentially all normal human peripheral T cells but not with normal human peripheral B cells, null cells, or macrophages.

5. Mouse complement-fixing monoclonal antibody which reacts with essentially all normal human peripheral T cells but not with normal human peripheral B cells, null cells, or macrophages.

6. A complement-fixing monoclonal antibody which reacts with essentially all normal human peripheral T cells but not with normal human peripheral B cells, null cells, or macrophages prepared by the method which comprises the steps of:

(i) immunizing mice with E rosette positive purified human T cells;

(ii) removing the spleens from said mice and making a suspension of the spleen cells;

(iii) fusing said spleen cells with mouse myeloma cells in the presence of a fusion promoter;

(iv) diluting and culturing the fused cells in separate wells in a medium which will not support the unfused myeloma cells;

(v) evaluating the supernatant in each well containing a hybridoma for the presence of antibody to E rosette positive purified T cells;

(vi) selecting and cloning a hybridoma producing antibody which fixes complement and reacts with essentially all normal human peripheral T cells but not with normal human peripheral B cells, null cells, or macrophages; and

(vii) recovering the antibody from the supernatant above said clones.

7. A complement-fixing monoclonal antibody which reacts with essentially all normal human peripheral T cells but not with normal human peripheral B cells, null

cells, or macrophages prepared by the method which comprises the steps of:

(i) immunizing mice with E rosette positive purified human T cells;

(ii) removing the spleens from said mice and making a suspension of the spleen cells;

(iii) fusing said spleen cells with mouse myeloma cells in the presence of a fusion promoter;

(iv) diluting and culturing the fused cells in separate wells in a medium which will not support the unfused myeloma cells;

(v) evaluating the supernatant in each well containing a hybridoma for the presence of antibody to E rosette positive purified T cells;

(vi) selecting and cloning a hybridoma producing antibody which fixes complement and reacts with essentially all normal human peripheral T cells but not with normal human peripheral B cells, null cells, or macrophages;

(vii) transferring said clones intraperitoneally into mice; and

(viii) harvesting the malignant ascites or serum from said mice, which ascites or serum contains the desired antibody.

8. A method of preparing complement-fixing monoclonal antibody which reacts with essentially all normal human peripheral T cells but not with normal human peripheral B cells, null cells, or macrophages, which comprises culturing the hybridoma ATCC CRL 8001 in a suitable medium and recovering the antibody from the supernatant above said hybridoma.

9. The monoclonal antibody prepared by the method of claim 8.

10. A method of preparing complement-fixing monoclonal antibody which reacts with essentially all normal human peripheral T cells but not with normal human peripheral B cells, null cells, or macrophages, which comprises injecting into a mouse the hybridoma ATCC CRL 8001 and recovering the antibody from the malignant ascites or serum of said mouse.

11. The monoclonal antibody prepared by the method of claim 10.

* * * * *

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Patrick Chung-Shu Kung and Gideon Goldstein
Serial No.	:	33,669
Filed	:	April 26, 1979
For	:	HYBRID CELL LINE FOR PRODUCING COMPLEMENT-FIXING MONOCLONAL ANTIBODY TO HUMAN T CELLS, ANTIBODY AND METHODS
Examiner	:	A. Fagelson
Group	:	125

Commissioner of Patents and Trademarks
Washington, D. C. 20231

TERMINAL DISCLAIMER UNDER 37 CFR 1.321

Dear Sir:

Ortho Pharmaceutical Corporation, Raritan, New Jersey, a corporation of New Jersey, represents that it is the Assignee of the entire right, title, and interest in the above-identified patent application by virtue of an unrecorded assignment executed by Patrick C. Kung and Gideon Goldstein on April 25, 1979, a copy of which is attached hereto.

Ortho Pharmaceutical Corporation hereby disclaims the terminal part of any patent granted on the above-identified application which would extend beyond the expiration of any United States Patent granted on copending application Serial No. 22,132, filed March 20, 1979, and hereby agrees that any patent so granted on the subject application shall be enforceable only for and during such period that the legal title to said patent shall be the same as the legal title to any patent issuing from application Serial No. 22,132, this agreement to run with any patent granted on the subject application and to be binding upon the grantee, its

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successors, or assigns.

Signed at Raritan, New Jersey this 1st day of July, 1981.

ORTHO PHARMACEUTICAL CORPORATION

BY: Glenn F. Kiplinger
Glenn F. Kiplinger,
Vice President
Research and Development

EXHIBIT C

PLA/ELA SUBMISSIONS

	SUBMISSIONS	ELA VOLUME	PLA VOLUME
I.	Original Submission March 29, 1984		
	Facilities, C.V.'s, Responsible Personnel	3.1	
	Blueprints of the facilities	3.2	
	Method of manufacture of ORT*3		1.1
	Explanation of the test methods/specifications		1.1
	All test methods divided into crude, purified, and final container		1.2
	Stability commitment and reports: PD 1332, PD 1450, PD 1476		1.2
	Labeling		1.3
	Clinical abstracts and overall summary		2.1
	Clinical summaries		2.2 - 2.4
	Appendicies and raw data		2.5 - 2.19
	Pre-clinical studies		2.20
	Dictionary of therapeutic classes of concomitant medication		2.21
	Literature references in alphabetical order by author		2.22 - 2.23
II.	Amendment 11/20/84 to OBRR Letter 10/9/84		
	CLinical Reponses (11 questions)		4.1
	Manufacturing responses (51 questions)		
	Questions 12-49		4.2
	Questions 50-56A		4.3
	Questions 56B-62		4.4
	Amendment		4.4
	Filed McNeil Janssen QC laboratory (In process testing)		
	Deleted DNA method		
	Information on Polysorbate 80		
	Revised raw material specifications		

PLA/ELA SUBMISSIONS

	SUBMISSIONS	ELA VOLUME	PLA VOLUME
III.	Amendment February 8, 1985 Additional Responses to OBRR Letter 10/9/84		
	Question 34, Appendix I - Coomassie Blue vs Silver Stained SDS Gels		5.1
	Question 38, Appendix I - IEP Gel of Purified ORT*3 vs Murine IgG, M, A		5.1
	Question 48, Appendix II - Stability Data [PDRR 1476-1 and PDRR 1616] - Stability Samples - Test Stations/Test Methods (Osmolality specification deleted)		5.1
	Amendment Appendix III (for ELA) New Resin Preparation Area (no validation data available)		5.1
IV.	Amendment February 20, 1985 to OBRR Verbal Request		
	Statistical Information (30 tables in B81-033 updated to 2 years post transplant/exception 1 yr infection data)		6.1 - 6.7
	Letter from Dr. Goldstein summarizing key safety and efficacy data for B81-033, efficacy for D83-038, pulmonary edema and infections through 12/31/84		6.1
V.	Amendment March 5, 1985 to OBRR Verbal Request February 20, 1985		
	Listing of all ORT*3 studies and status, Appendix I.		7.1
	Slides on indications and incidence of severe pulmonary edema, Appendix II		7.1
	Life Table Analyses B81-033 (Kidney and patient survival), Appendix III		7.1
	Dr. Starzl's publication on B-cell lymphomas, Appendix IV		7.1
	Pooled Comparison of Adverse Reactions (0-45) for ORT*3 vs STEROIDS for B81-033, Appendix V		7.1
	Testing results of ORT*3 pristane residuals, Appendix VI		7.1

PLA/ELA SUBMISSIONS

	SUBMISSIONS	ELA VOLUME	PLA VOLUME
VI.	Submission to the Statistician of Information Supplied March 5, 1985 (Duplicate information)		
	Patient and Kidney Survival Graphs (B81-033)		8.1
	Incidence bar graph of adverse experiences (B81-033)		8.1
VII.	Amendment March 27, 1985 to OBRR letter 10/9/84		
	Question 19		9.1
	Process validation of ORTHOCLONE OKT*3		
	Autoclave, dry heat oven, media fill studies, calibration, analytical and biological challenge reports from McNeil/Janssen		
VIII.	Amendment June 18, 1985 Response to OBRR Letter 4/24/85		
	(Proposed generic name for ORTHOCLONE OKT*3 as murine moab anti CD3 (human T cell blocker), Cover Letter		10.1
	Clinical Questions 1-8 (data lockpoint 12/31/84) (B81-033, D83-038, D83-069, and C82-068)		10.1
	Kidney + patient survival curves, adverse reaction bar graphs supplementing the 8 questions in Volume 10.1		10.2
	Manufacturing questions 9 - 20		10.3
	Manufacturing questions 31 - 54		10.4
	- Stability questions, protein aggregates, questions on validation data		
	Amendment		
	For Ortho Q.A. laboratory, production personnel, proposed marketed stability program		10.4
IX.	Amendment September 18, 1985 Responses to OBRR letter 8/20/85		
	Statistical analysis of patient data outlining 16 parameters for studies B81-033, D83-038, D83-069 and C82-068		11.1 - 11.2
X.	Submission October 7, 1985 to OBRR Letter 4/24/85		
	Amino-Terminal Sequence H & L Chain		12.1
	All Testing Methods and Specifications		12.1
	Revised Package Insert		12.2
	Amendment		
	For the ASF Laboratory with Blueprints		12.3

PLA/ELA SUBMISSIONS

	SUBMISSIONS	ELA VOLUME	PLA VOLUME
XI.	Submission November 1, 1985 to OBRR Letter 10/18/85		
	Clinical Question 1 (Post-Marketing)		14.1
	Manufacturing and Control Questions 2-18		14.1
	-DNA		
	-Stability		
	-Filter Study		
	DCCA Plots and SDS Photographs		14.2
XII.	Submission December 6, 1985		
	Viral test results of hybridoma cell line		15.1
	Test results on selected harvests of ascitic fluid and purified lots of ORT*3		15.1
	Viral testing on ORT*3 with corresponding animal information and test volumes		15.1
	Microbiological profile of environmental and animal health monitoring conducted at Jackson Laboratories		15.1
	Residual Testing results for pristane, calf serum and horse serum		15.1
	Description of inspection of incoming ampules for ORT*3		15.1
	Reassay procedure for ORT*3 test method		15.1
XIII.	Amendment 1/16/86 to OBRR letter of 12/12/85		
	Responses to 23 questions		
	- Appendix I, a proposed validation protocol for removing Rauscher's Murine Leukemia Virus from ORT*3 ascitics fluid by the ORT*3 purification process		16.1
	- Appendix III, diagrams of the Monoclonal Antibody Production Complex and QA facilities at Ortho and Janssen/McNeil Parenteral Facility		16.1
	- Request for 16 month expiration dating		16.1
	Validation, Protocol, and Equipment Installation/ Qualification data for the dry heat oven.		16.2
XIV.	Amendment 1/31/86 to OBRR Request 1/16/86		17.1
	Responses to 7 additional questions		
	Appendix I - SOP for equipment depyrogenation		17.1
XV.	Submission March 13, 1986		N/A
	- Response to Pre-License Inspection March 4 through 6, 1986		

PLA/ELA SUBMISSIONS

	SUBMISSIONS	ELA VOLUME	PLA VOLUME
XVI.	Amendment 4/7/86 to OBRR Comments of 4/2/86 - Appendix I - Form FDA 2567 - Appendix II - Revised ORTHO immediate container label Revised ORTHO package label - Appendix III - Revised ORTHO package circular - Appendix IV - OBRR package circular revisions requested in April 2, 1986 transmittal. - Appendix V - New references suggested for inclusion in package circular by OBRR		18.1
XVII.	Amendment 4/10/86 to OBRR Telecommunication of April 2, 1986 by Dr. K. Mittal - IND Protocols - ORTHO Commitment with OBRR (post-approval)		19.1
XVIII.	Amendment 4/22/86 to OBRR Request 4/18/86 - Appendix I - Labeling - Appendix II - Responses and Commitments - Appendix III - Mycoplasma		20.1
XIX.	Export Amendment 5/7/86 to OBRR Request 4/7/86 - Flow Chart - Details of Shipping Carton & Tests Conducted - Release Protocol - Revised Shipping Label		21.1
XX.	Submission 5/29/86 to Dr. Weng's Request Analysis of the statistical method, Breslow's generalized Wilcoxon test, utilized in analyzing 2 year kidney survival times for Study 881-033.		22.1
XXI.	Submission 6/11/86 to OBRR Revisions Received on 6/10/86 - Appendix I - Revised package circular per OBRR copy received June 10, 1986. - Appendix II - Reversal rate and percentages for rescue data in 225 patients. - Appendix III - Xerox copies of Dr. Starzl's figures and legends from his paper, "Use of ORT*3 with Cyclosporine and Steroids for Acute Kidney and Liver Allograft Rejection" requested by Dr. Mittal. - Appendix IV - Literature references on Polysorbate 80.		23.1
XXII.	Submission 6/16/86 to OBRR - Revisions Received 6/16/86 Package Insert Revisions		24.1

ORTH 345 PATENT

EXHIBIT D

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors	:	Patrick C. Kung and Gideon Goldstein
U.S. Patent No.	:	4,361,549
Issued	:	November 30, 1982
For	:	COMPLEMENT-FIXING MONOCLONAL ANTIBODY TO HUMAN T CELLS, AND METHODS OF PREPARING SAME

POWER OF ATTORNEY

AND

APPOINTMENT OF AGENTS

ORTHO PHARMACEUTICAL CORPORATION, owner of the entire right, title and interest in and to the above-identified patent through its duly authorized officer hereby appoints the following attorneys to prosecute an application pursuant to 35 U.S.C. 156 for extension of the term of the above-identified patent and to act as its agents in respect of said application: Leonard P. Prusak, (Reg. #18,059), Robert L. Minier (Reg. #20,038), Wayne R. Eberhardt (Reg.#22,804), Audley A. Ciamporcero, Jr. (Reg.#26,051) and Geoffrey G. Dellenbaugh (Reg. #26,864).

Kindly send all correspondence in connection with this matter to:

Leonard P. Prusak
Johnson & Johnson
One Johnson & Johnson Plaza
New Brunswick, NJ 08933-7003

ORTH 345 PATENT

EXHIBIT E

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors : Patrick C. Kung and Gideon Goldstein

U.S. Patent No.: 4,361,549

Issued: : November 30, 1982

For : COMPLEMENT-FIXING MONOCLONAL ANTIBODY TO HUMAN T CELLS, AND METHODS OF PREPARING SAME

DECLARATION

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

I, GEOFFREY G. DELLENBAUGH, residing at 117 South Main Street, Pennington, New Jersey, 08534, declare as follows:

1) THAT I am a Patent Attorney authorized to practice before the United States Patent and Trademark Office and that my registration number is 26,864.

2) THAT I make this Declaration as the agent of Ortho Pharmaceutical Corporation, a corporation of New Jersey.

3) THAT I have reviewed and understand the contents of the Application for Patent Term Extension which is submitted pursuant to 35 U.S.C. 156, of which the present Declaration is attached as EXHIBIT E.

4) THAT I believe that U.S. patent 4,361,549 is subject to extension pursuant to Section A of the Guidelines for Extension of Patent Term under 35 U.S.C. 156 published October 9, 1984 at 1047 Official Gazette 16.

Kindly address all telephone calls to Geoffrey G. Dellenbaugh (Reg. #26,864) at telephone number (201) 545-5545.

ORTHO PHARMACEUTICAL CORPORATION

Benjamin F. Lambert

Benjamin F. Lambert

August 13, 1986
Date

Assistant Secretary
Title

5) THAT I believe an extension of two hundred one (201) days of the term of the U.S. Patent 4,361,549 is fully justified under 35 U.S.C. 156.

6) THAT I believe U.S. Patent 4,361,549 for which the extension is being sought meets the conditions for extension of the term of a patent as set forth in 35 U.S.C. 156 and Section B of the Guidelines for Extension of Patent Term under 35 U.S.C. 156 published October 9, 1984 at 1047 Official Gazette 16.

I hereby declare that all statements made herein of my own knowledge are believed true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Seciton 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application and any extension of U.S. Patent 4,361,549.

Date: August 12, 1986

Geoffrey G. DeVenbaugh

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